

Chromosomal characterization and relationship between two new species of *Ctenomys* (Rodentia, Ctenomyidae) from northern Córdoba province, Argentina

By MABEL D. GIMÉNEZ, C. J. BIDAU, CARINA F. ARGÜELLES, and J. R. CONTRERAS

Laboratorio de Genética Evolutiva, Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones, Posadas, Misiones, Argentina

Receipt of Ms. 06. 03. 1998

Acceptance of Ms. 14. 10. 1998

Abstract

Karyotypes of two recently described species of *Ctenomys* from northern Córdoba province (Argentina) were studied. *C. osvaldoreigi* is only known from the type locality in the high valleys of the Sierras Grandes at more than 2000 m above sea level. The karyotype consists of $2n = 52$ chromosomes with $FN = 56$ and includes 22 pairs of telocentric autosomes that decrease gradually in size, a pair of subtelocentric autosomes ($n^{\circ}8$), two pairs of small metacentrics and a pair of sex chromosomes. Three populations from the northeastern plains of Córdoba province (including one from the type locality) of *C. rosendopascuali* were analyzed. All individuals were $2n = 52$ but FN s of the three populations were different. Individuals from Los Mistoles showed $FN = 62$ and the karyotype consists of a large subtelocentric autosomal pair, a medium-sized subtelocentric ($n^{\circ}8$), twenty telocentric and three small metacentric pairs plus a pair of sex chromosomes. Candelaria specimens had $FN = 64$; the karyotype includes a second large subtelocentric pair which replaces a large telocentric, the remainder of the complement being similar to Los Mistoles. A further large subtelocentric occurs in the Mar Chiquita population, thus $FN = 66$; the remainder of the karyotype does not differ from the two other populations. In order to compare the new species to a known species of the same general geographical area, four populations of *C. bergi* from northwestern Córdoba were karyotyped. All specimens had $2n = 48$, $FN = 90$. The three karyotypes found in *C. rosendopascuali* are remarkably similar and obviously related to that of *C. osvaldoreigi* through relatively simple chromosomal rearrangements, which confirms their morphological and molecular proximity.

Key words: *Ctenomys rosendopascuali*, *C. osvaldoreigi*, *C. bergi*, northern Córdoba, karyotype

Introduction

The South American Octodontoidea are a remarkable group of mammals with respect to their extraordinary karyotypic diversity. Diploid chromosome numbers range from $2n = 10$ in the Bolivian species *Ctenomys steinbachi* (Ctenomyidae) (ANDERSON et al. 1987; RUEDAS et al. 1993) to $2n = 102$ in *Tympanoctomys barrerae* (Octodontidae) (CONTRERAS et al. 1990). Fundamental numbers (FN) also vary enormously (16–202). Most of this chromosomal diversity is due to karyotypic variation within a single genus: *Ctenomys* (BIDAU et al. 1996; CONTRERAS et al. 1990; GIMÉNEZ et al. 1997; ORTELLS 1995; ORTELLS et al. 1990; REIG et al. 1990, 1992).

Ctenomys, with more than 60 extant species, is one of the best examples of “explosive” speciation accompanied by extensive karyotype repatterning (BIDAU et al. 1996;

REIG 1984, 1989; REIG et al. 1990). According to fossil data, the *Ctenomys* radiation is thought to have occurred 1.8 MY ago (ORTELLS 1990; REIG et al. 1990). These evidences strongly suggest that the main mode of speciation has been (and is) chromosomal. The subterranean mode of life plus the populational characteristics of most of the species (small deme size, low vagility) support the chromosomal speciation hypothesis (BIDAU et al. 1996; KING 1993).

In this study we investigate karyotypes of *Ctenomys* from northern Córdoba province (Argentina). The analyzed populations belong to two new biological species, *C. rosendopascuali* and *C. osvaldoreigi* (CONTRERAS 1995 a, b). Our results are compared to previous ones and discussed within the frame of a model for the evolution of the genus which incorporates molecular, morphological, and paleobiogeographical data.

Material and methods

This work is based on the individuals of *Ctenomys* indicated in table 1 and figure 1. All specimens were deposited in the collection of the PROBBAS (CONICET, Corrientes, Argentina), with the following catalogue numbers (sex in parentheses): *C. rosendopascuali*. Mar Chiquita: C-03363 (F), C-03364 (M). Candelaria: C-03464 (M), C-03465 (F). Los Mistoles: C-03509 (M), C-03510 (F). *C. osvaldoreigi*. Estancia San Luis (Sierras Grandes): C-03462 (F), C-03463 (F), C-03977 (F), C-03978 (F), C-03979 (F), C-03980 (F). *C. bergi*. Cruz del Eje: C-03460 (M), C-03461 (M). Las Toscas: C-03506 (M). Salinas Grandes: C-03507 (F). Guanaco Muerto: C-03508 (F).

Mitotic metaphases were obtained following two protocols: direct bone-marrow preparations according to a modified version of FORD and HAMERTON's (1956) technique, and short-term bone-marrow in vitro culture (GIMENEZ and BIDAU 1994). In the first case, bone marrow was incubated in 0.1 ml 0.05 % colchicine plus 9.9 ml 0.075 M KCl for 55 min at 37°C and subsequently fixed in 3:1 methanol:glacial acetic acid. For short-term culture, the tissue was incubated in RPMI 1640 medium supplemented with 15 % foetal calf serum for 20.5 h at 37°C. A drop of 0.005 % colchicine was then added to the culture, and 15 min later the cells were hypotonized in 0.075 M KCl and fixed in 3:1. Nondifferential chromosome staining was performed in phosphate buffered Giemsa (pH = 6.8). G- and C-banding followed the protocols of SEABRIGHT (1971) and SUMNER (1972), respectively. NORs were stained according to HOWELL and BLACK (1980). Meiotic preparations for the observation of sperm morphology were made by the technique of EVANS et al. (1964).

Table 1. Localities, number and sex of the *Ctenomys rosendopascuali*, *C. osvaldoreigi* and *C. bergi* individuals studied.

Locality		N° of specimens	
		Male	Female
<i>C. rosendopascuali</i> Contreras, 1995			
Mar Chiquita ¹	(30°55' S–62°41' W)	1	1
Candelaria	(29°49' S–63°21' W)	1	1
Los Mistoles	(30°38' S–63°54' W)	1	1
<i>C. osvaldoreigi</i> Contreras, 1995			
Estancia San Luis ¹ (Sierras Grandes)	(31°24' S–64°48' W)	–	6
<i>C. bergi</i> Thomas, 1902			
Cruz del Eje ¹	(30°44' S–64°48' W)	1	1
Guanaco Muerto	(30°27' S–65°01' W)	–	1
Salinas Grandes	(30°03' S–65°05' W)	–	1
Las Toscas	(30°08' S–64°53' W)	1	–

¹ Type localities

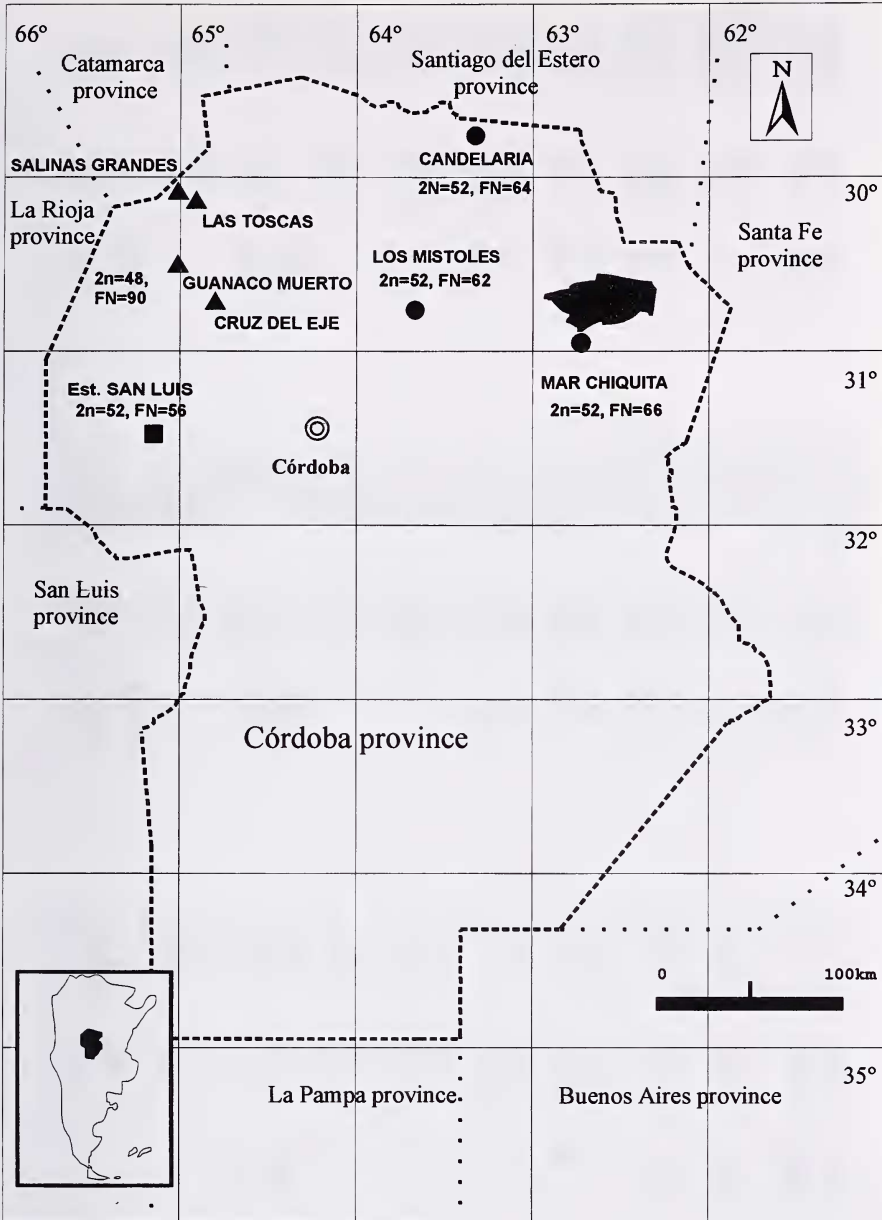
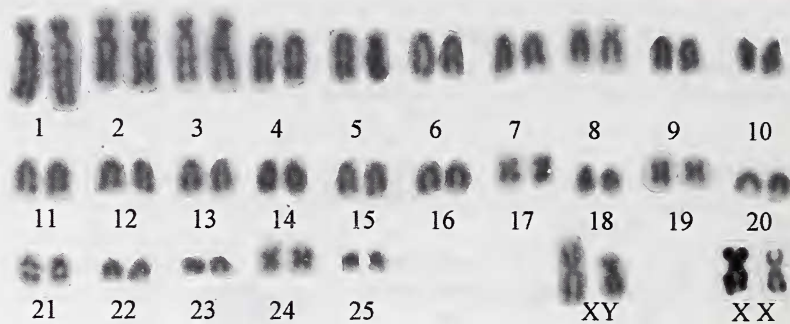
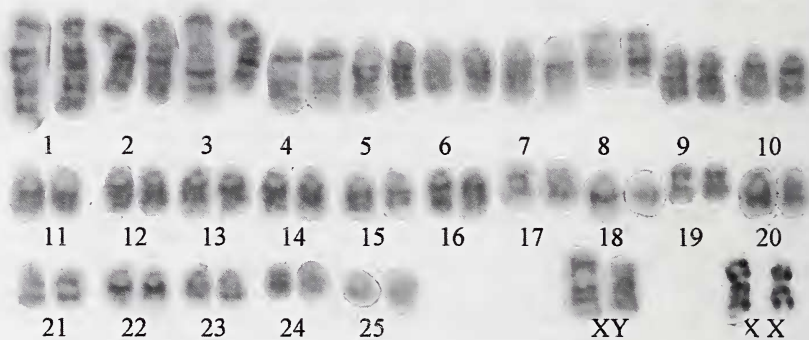


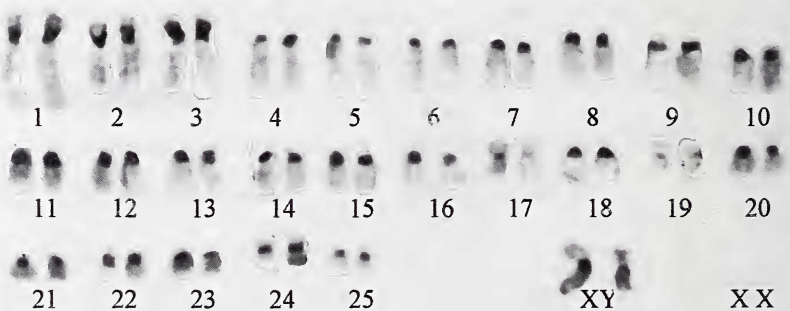
Fig. 1. Geographic distribution of the populations of *Ctenomys rosendopascuali*, *C. osvaldoreigi*, and *C. bergi* analyzed.



a

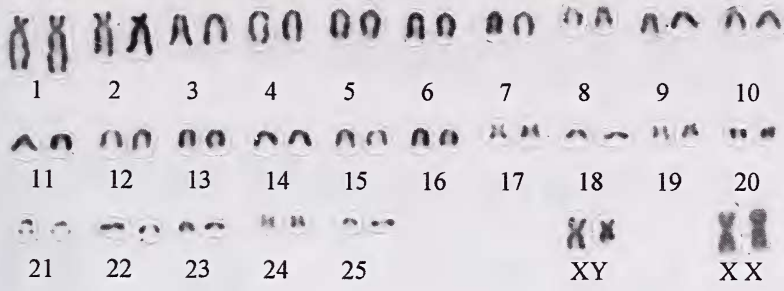


b

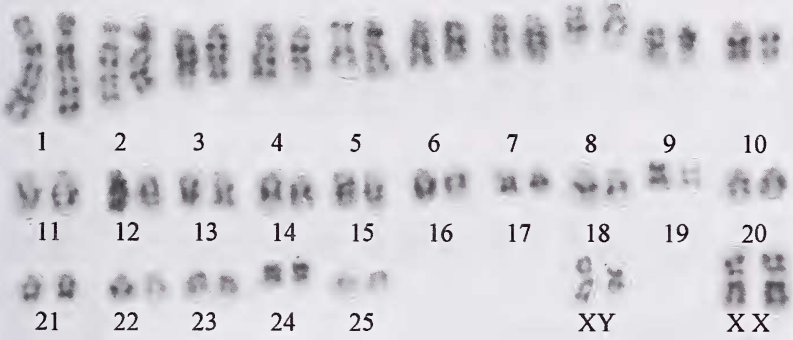


c

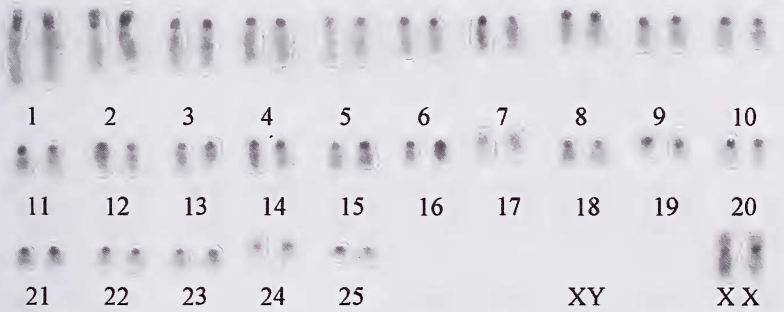
Fig. 2. Karyotype of *C. rosendopascuali* from Mar Chiquita; a. Giemsa stained, b. G-banding, c. C-banding. Bar = 10 μ m.



a



b



c

Fig. 3. Karyotype of *C. rosendopascuali* from Candelaria; a. Giemsa stained, b. G-banding, c. C-banding. Bar = 10 μ m.

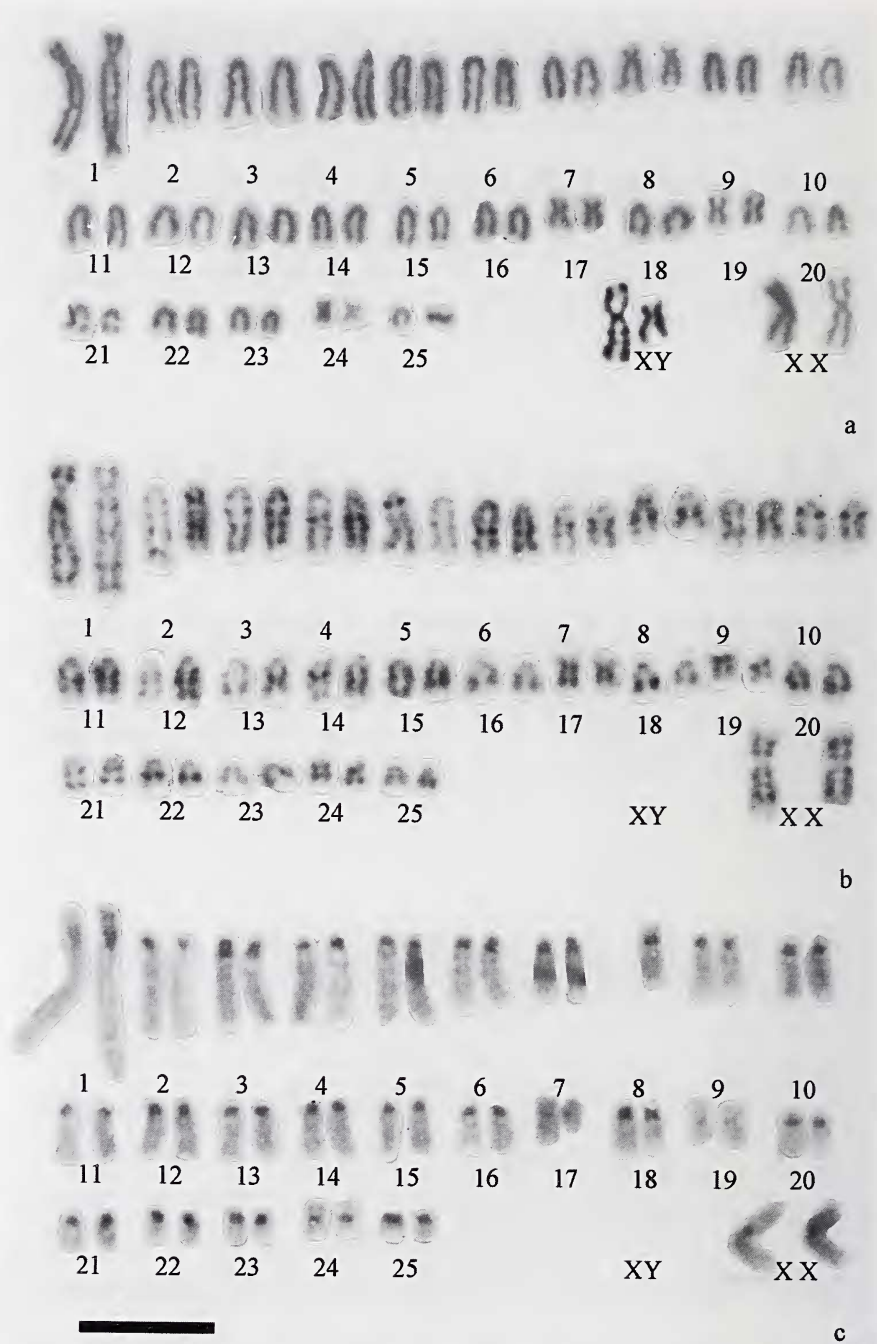


Fig. 4. Karyotype of *C. rosendopascuali* from Los Mistoles; a. Giemsa stained, b. G-banding, c. C-banding. Bar = 10 μ m. (Because of technical problems during the production process the second No. 8 chromosome in the third line from bottom is missing!)

Results

C. rosendopascuali

The karyotype of the specimens from Mar Chiquita (the type locality) consists of $2n = 52$ chromosomes: pairs 1 to 3 are large subtelocentric autosomes; pairs 4–7, 9–16, 18, 20–23, and 25 are telocentric; the marker pair number 8 is subtelocentric. Pair 21 has an interstitial secondary constriction that represents the single NOR. Pairs 17, 19, and 24 are three small biarmed elements (Figs. 2, 3, 4). The X chromosome is metacentric and represents 7% of the haploid genome; the Y chromosome is metacentric and small (Fig. 2). FN is thus 66.

Heterochromatin distribution is para- or pericentromeric with prominent C-bands. Pairs 1 to 3 show partially C-positive short arms (Fig. 2 c). Silver impregnation demonstrated that the secondary constriction of pair 19 corresponds to an active interstitial NOR (Fig. 6 a).

The chromosome complement of the animals from Candelaria is basically similar; they are $2n = 52$ but instead of 3 pairs of large subtelocentric autosomes only two occur, thus, the FN is reduced to 64 (Fig. 3). A further reduction of the FN to 62 chromosome arms occurs in the $2n = 52$ individuals from Los Mistoles which otherwise have a similar karyotype to Mar Chiquita and Candelaria (Fig. 4). Table 2 shows a comparison of relative lengths and centromeric indexes of the three karyotypes.

In the three samples, the distribution of heterochromatin in the autosomes and sex-chromosomes was basically similar. The X-chromosome had a centromeric band while the Y-chromosome showed a uniform intermediate staining. The large biarmed autosomes showed positive C-banding in the pericentromeric region which extended partially to the short arms while the remainder of the autosomes exhibited prominent C-bands in the para- or pericentromeric regions (Figs. 2 c, 3 c, 4 c). Sperm is of the simple asymmetric type.

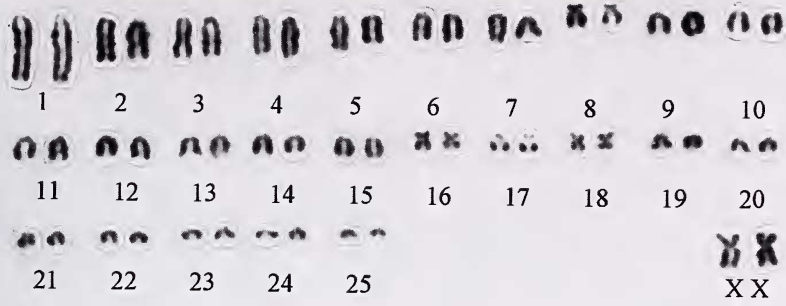
Table 2. Relative length (RL) and centromeric index (CI) of the chromosomes of four analysed populations of *Ctenomys* from Córdoba. In parentheses, chromosome morphology according to the nomenclature of LEVAN et al. (1964)

CN°	<i>C. rosendopascuali</i>						<i>C. osvaldoreigi</i>	
	Mar Chiquita		Candelaria		Los Mistoles		Ea. San Luis	
	RL	CI	RL	CI	RL	CI	RL	CI
1	9.05 ±0.42	22.56 ±0.69 (st)	9.94 ±0.22	20.87 ±1.38 (st)	10.56 ±0.54	21.87 ±0.68 (st)	11.23 ±0.20	0 (t)
2	6.77 ±0.31	28.36 ±0.64 (sm)	7.67 ±0.29	20.68 ±1.46 (st)	6.55 ±0.22	0 (t)	6.99 ±0.09	0 (t)
3	6.75 ±0.32	24.24 ±0.98 (st)	5.82 ±0.11	0 (t)	5.85 ±0.09	0 (t)	6.49 ±0.08	0 (t)
4	5.33 ±0.10	0 (t)	5.67 ±0.04	0 (t)	5.70 ±0.10	0 (t)	6.16 ±0.13	0 (t)
5	5.10 ±0.10	0 (t)	5.27 ±0.12	0 (t)	5.38 ±0.15	0 (t)	5.29 ±0.23	0 (t)
6	4.28 ±0.13	0 (t)	4.59 ±0.11	0 (t)	4.80 ±0.09	0 (t)	4.68 ±0.08	0 (t)
7	4.25 ±0.10	0 (t)	4.29 ±0.08	0 (t)	4.10 ±0.13	0 (t)	4.39 ±0.05	0 (t)
8	4.30 ±0.29	18.73 ±0.98 (st)	4.07 ±0.06	19.97 ±1.05 (st)	3.70 ±0.07	15.28 ±0.33 (st)	4.06 ±0.13	16.65 ±1.16 (st)

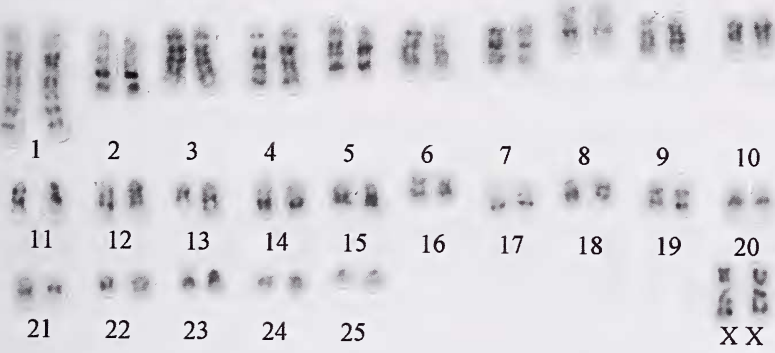
Table 2. Continued.

CN°	<i>C. rosendopascuali</i>						<i>C. osvaldoreigi</i>	
	Mar Chiquita		Candelaria		Los Mistoles		Ea. San Luis	
	RL	CI	RL	CI	RL	CI	RL	CI
9	3.95 ±0.14	0 (t)	3.92 ±0.06	0 (t)	3.63 ±0.07	0 (t)	4.04 ±0.09	0 (t)
10	3.68 ±0.10	0 (t)	3.66 ±0.05	0 (t)	3.57 ±0.07	0 (t)	3.82 ±0.09	0 (t)
11	3.47 ±0.09	0 (t)	3.47 ±0.08	0 (t)	3.52 ±0.08	0 (t)	3.69 ±0.06	0 (t)
12	3.26 ±0.14	0 (t)	3.43 ±0.07	0 (t)	3.40 ±0.06	0 (t)	3.52 ±0.05	0 (t)
13	3.26 ±0.15	0 (t)	3.28 ±0.08	0 (t)	3.31 ±0.05	0 (t)	3.19 ±0.10	0 (t)
14	3.14 ±0.10	0 (t)	3.24 ±0.09	0 (t)	3.28 ±0.04	0 (t)	3.04 ±0.11	0 (t)
15	3.13 ±0.10	0 (t)	3.11 ±0.08	0 (t)	3.12 ±0.06	0 (t)	2.94 ±0.08	0 (t)
16	2.87 ±0.10	0 (t)	2.95 ±0.07	0 (t)	2.99 ±0.07	0 (t)	2.85 ±0.23	41.85 ±1.08 (m)
17	2.87 ±0.06	41.67 ±2.41 (m)	2.49 ±0.12	45.72 ±1.53 (m)	2.51 ±0.14	40.71 ±0.56 (m)	2.74 ±0.05	0 (t)
18	2.79 ±0.13	0 (t)	2.67 ±0.11	0 (t)	2.80 ±0.13	0 (t)	2.44 ±0.10	45.00 ±1.87 (m)
19	2.56 ±0.06	43.24 ±2.04 (m)	2.29 ±0.15	47.12 ±0.64 (m)	2.41 ±0.11	43.92 ±1.56 (m)	2.31 ±0.10	0 (t)
20	2.52 ±0.13	0 (t)	2.36 ±0.14	0 (t)	2.55 ±0.04	0 (t)	2.17 ±0.09	0 (t)
21	2.35 ±0.12	0 (t)	2.36 ±0.10	0 (t)	2.45 ±0.09	0 (t)	2.06 ±0.06	0 (t)
22	2.30 ±0.11	0 (t)	2.06 ±0.07	0 (t)	2.20 ±0.09	0 (t)	1.76 ±0.04	0 (t)
23	2.18 ±0.12	0 (t)	1.93 ±0.07	0 (t)	1.97 ±0.02	0 (t)	1.69 ±0.05	0 (t)
24	2.14 ±0.06	47.96 ±0.24 (m)	2.27 ±0.13	47.48 ±0.27 (m)	1.96 ±0.09	44.28 ±1.63 (m)	1.60 ±0.08	0 (t)
25	2.00 ±0.07	0 (t)	1.50 ±0.05	0 (t)	1.55 ±0.03	0 (t)	1.36 ±0.04	0 (t)
X	5.96 ±0.49	41.39 ±1.32 (m)	5.67 ±0.19	39.52 ±1.09 (m)	6.16 ±0.26	39.09 ±0.85 (m)	5.56 ±0.14	36.66 ±1.85 (sm)
Y	4.11 ±0.14	29.62 ±2.25 (sm)	3.48 ±0.26	32.52 ±0.57 (sm)	3.73 ±0.15	32.43 ±0.67 (sm)	*	*

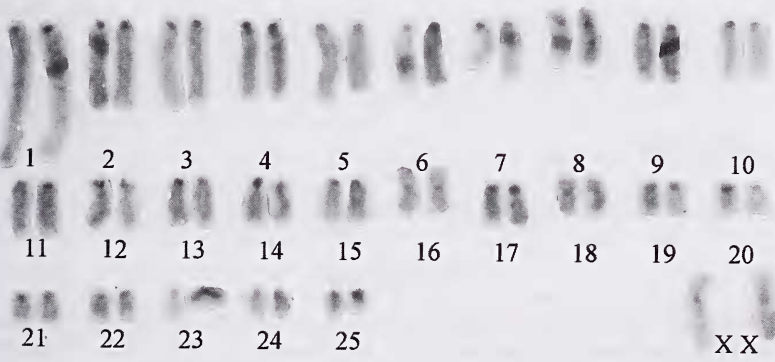
Notes: CN° = chromosome number. Relative length (RL) was calculated as the length percentage of each chromosome pair per haploid complement. Centromeric Index (CI) was calculated according to LEVAN et al. (1964) as Length of Short Arm × 100/Total Chromosome Length. Thus chromosomes are classified as m if CI = 50–37.5, sm if CI = 37.5–25.0, st if CI = 25.0–12.5 and t if CI = 12.5–0. In all cases, Standard Error is indicated. * No information is available on the Y chromosome of *C. osvaldoreigi*.



a



b



c

Fig. 5. Karyotype of *C. osvaldoreigi* from Estancia San Luis; a. Giemsa stained, b. G-banding, c. C-banding. Bar = 10 μ m.

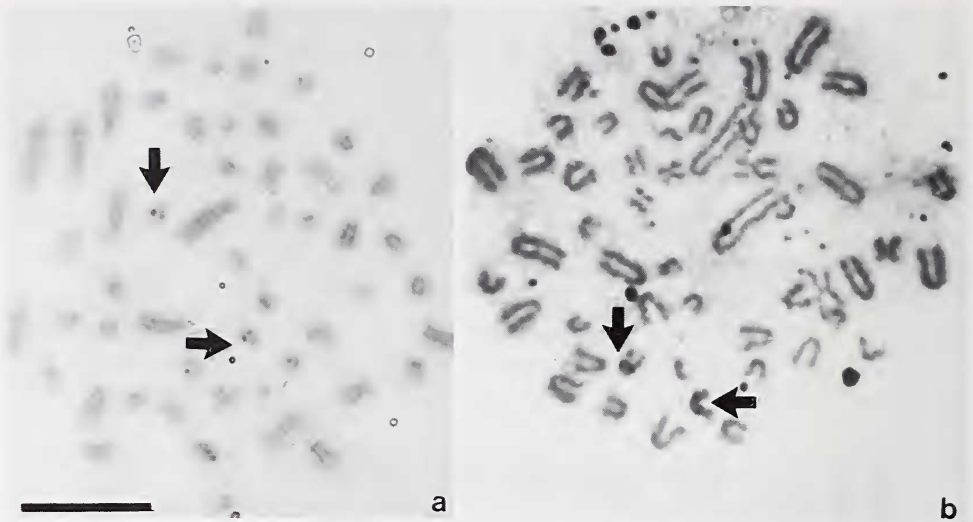


Fig. 6. Silver staining of NORs in (a) *C. rosendopascuali* from Mar Chiquita and (b) *C. osvaldoreigi*. Arrows indicate the NOR carrying autosomes. Bar = 10 µm.

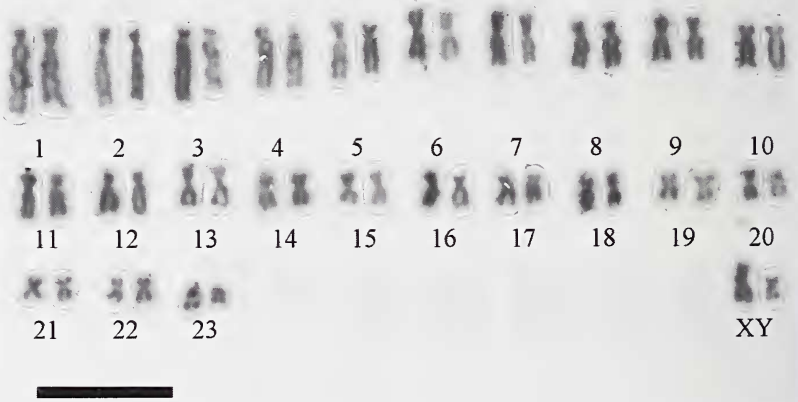


Fig. 7. Giemsa stained karyotype of *Ctenomys bergi* from Cruz del Eje. Bar = 10 µm.

C. osvaldoreigi

The specimens from the Sierras Grandes were collected at more than 2000 m above sea level at Estancia San Luis (the type locality). The karyotype of these individuals is very similar to the previously described forms (Fig. 5) and consists of seven pairs of telocentric autosomes of decreasing size, the marker subtelocentric pair n°8, telocentric pairs 9 to 15, 17, and 19–25 which decrease gradually in size and two small metacentric pairs: 16 and 18 (Tab. 2). Pair 17 carries the single NOR but in this case, the secondary constriction is procentric (Fig. 6b) which differentiates it from the other specimens in which the NOR is interstitial. The X chromosome is a large metacentric. Since no males were karyotyped, no information on the Y chromosome is available. C-banding revealed that the chromosomes have a small amount of centric constitutive heterochromatin (Fig. 5c). Sperm is of the simple asymmetric type.

C. bergi

In order to compare the karyotypes of the new species with other forms of the general geographic area, we examined samples from four populations of *C. bergi* from northwestern Córdoba province, including the type locality. The karyotype of this species had already been described by REIG et al. (1990) from a single locality. Our results confirmed the published karyotype of $2n = 48$ and $FN = 90$ which consists of 22 pairs of biarmed autosomes of different morphologies and a single telocentric pair which carries the NOR; both the X and the Y chromosomes are metacentric (Fig. 7). A number of short arm heteromorphisms was detected, probably due to the heterochromatic nature of these arms, but they will be described elsewhere. This species has a symmetric sperm.

Discussion

Ctenomys includes more than 60 species distributed through an extensive area of South America. The amount of knowledge already accumulated including morphological, biogeographical, paleontological, chromosomal, genetic, and molecular data (BIDAU et al. 1996; CONTRERAS, 1996; GIMÉNEZ et al. 1996; MIROL et al. 1995 a, b; ORTELLS, 1990, 1995; REIG et al. 1990) allows the formulation of preliminary interpretations of its evolutionary history that dates back to the Pliocene (CONTRERAS et al. 1997). An ancestral stock which evolved in and expanded from the northern highlands of Bolivia and Perú is considered. *C. opimus* would be the closer extant form to the ancestral stock. From the latter, a number of species that occupy the area of the ancestral stock, evolved. These forms retain a certain degree of plesiomorphism and include: *C. frater*, *C. tuconax*, *C. scagliai*, *C. knighti* (all with symmetric sperm) and *C. osvaldoreigi* (with simple asymmetric sperm). The same primitive stock gave rise along its southward expansion and differentiation to the so-called "chacoan" and "parachacoan" species. The first of these branches consists of *C. boliviensis*, *C. goodfellowi*, *C. nattereri*, and *C. rondoni* (with symmetric sperm). A closely related species sequence includes *C. sp.* (from Chuquisaca, Bolivia), *C. conoveri* and *C. sp.* (from Eastern Paraguay), all of them with a tendency towards gigantism and symmetric sperm. Further south, another sequence includes *C. scagliai*, *C. tucumanus*, *C. occultus*, *C. latro*, *C. argentinus*, and *C. pilarensis*, also with symmetric sperm. In "parachacoan" areas and originating from *C. osvaldoreigi* from the Sierras Grandes (Córdoba province, Argentina) derives the *C. rosendopascuali* sequence of the northern plains of Córdoba, and *C. yolandae* from Santa Fe which in turn is related with a complex of mesopotamic and Uruguayan species and with *C. bonettoi* that expands northwards towards the Chaco (GIMÉNEZ et al. 1996, 1997). All the latter forms have simple asymmetric sperm.

There is a close chromosomal relationship between *C. osvaldoreigi* and *C. rosendopascuali* which is in agreement with their morphological affinities (CONTRERAS 1995 a, b). In fact, only three fixed karyotypic differences occur between both species: 1) The position of the secondary constriction in the chromosome pair carrying the NOR, which is proximal in *C. osvaldoreigi* and interstitial in *C. rosendopascuali* and perhaps due to a paracentric inversion; 2) The existence of a third small metacentric autosomal pair in *C. rosendopascuali*; and 3) The biarmed nature of chromosome $n^{\circ}1$ in the three analyzed populations of the latter species. The other two chromosomal differences are polytypic and probably of more recent origin. Chromosomal polytypism and polymorphism are not infrequent in *Ctenomys* and have been reported in very different species involving many kinds of chromosomal rearrangements (BIDAU et al. 1996; GIMÉNEZ et al. 1996, 1997; ORTELLS 1995; ORTELLS et al. 1990; FREITAS 1994). This chromosomal variation is a small-scale reflection of the general situation of the genus, in which chromosomal speciation has probably been central to its "explosive" radiation (BIDAU et al. 1996;

REIG et al. 1990). Thus, chromosomal polytypisms could represent potential incipient stages of chromosomal speciation (GIMÉNEZ et al. 1997). It must be noted, however, that different classes of chromosomal rearrangements have widely different effects on heterozygote fertility and thus, their involvement in reproductive isolation and speciation must be regarded cautiously (BIDAU 1991; CONTRERAS et al. 1990; KING 1993; WHITE 1973, 1978).

The origin of the large biarmed chromosomes of *C. rosendopascuali* can be interpreted, on the basis of G- and C-band homologies, as a sequence of rearrangements start-

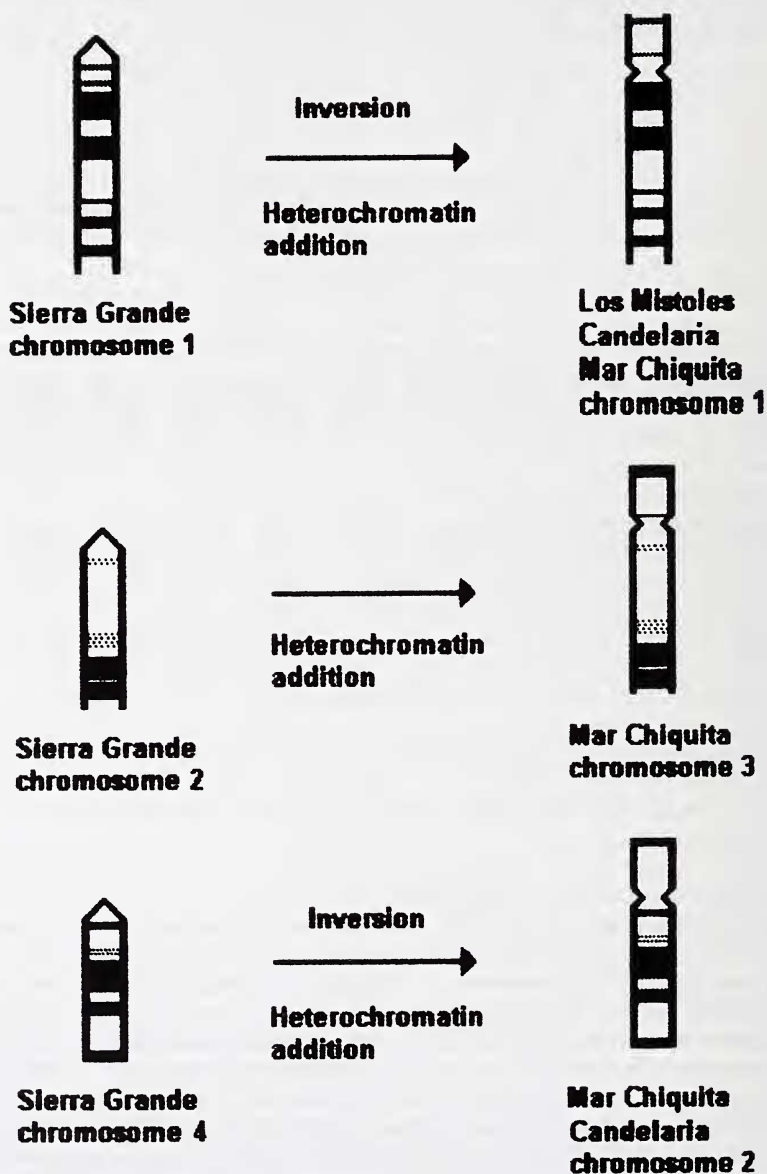


Fig. 8. Main chromosomal relationships between *Ctenomys rosendopascuali* and *C. osvaldoreigi*.

ing with the karyotype of *C. osvaldoreigi* (Fig. 8) which is assumed as ancestral according to the morphological evidence (CONTRERAS 1995 a, b; CONTRERAS et al. 1990). Pair n° 1 of this species, which is present throughout its whole range, originated through a pericentric inversion and increase in heterochromatin content. Pair n° 2, present in the Mar Chiquita and Candelaria populations, also arose by a pericentric inversion and heterochromatin addition from the standard chromosome n° 4 of *C. osvaldoreigi*. Pair n° 3 of the Mar Chiquita individuals is restricted to this population and is probably of recent origin; it arose by heterochromatin addition that produced the short arm, from pair n° 2 of *C. osvaldoreigi*. Karyotypic evolution through heterochromatin variation seems to be a common mechanism in *Ctenomys* (FREITAS 1994; MASSARINI et al. 1991) although its meaning is still obscure.

The study of *C. osvaldoreigi* and *C. rosendopascuali* poses a number of questions regarding the general relationships of the *Ctenomys* species. First, it led us to question the validity of karyotypic comparisons for the detection of evolutionary relationships within the genus. It is true that both species were originally identified by the unique character of their karyotypes which led to a detailed taxonomic study and their description as new species (CONTRERAS 1995 a, b). However, the chromosomal relationship of the more pleiomorphic form, *C. osvaldoreigi*, with the species to which it is taxonomically closer, is obscure. For example, *C. tuconax* is $2n = 58-61$, $FN = 80$; *C. scagliai* is $2n = 36$, $FN = 64$; *C. opimus* is $2n = 26$, $FN = 48$ (ORTELLS 1990) and *C. frater*, $2n = 52$, $FN = 78$ (COOK et al. 1990). Although detailed comparisons of banded karyotypes (which are in progress) are needed, this small sample of chromosomal variation is a clear indication of the difficulties involved in this type of analysis. Furthermore, the lack of knowledge about the ancestral karyotype of the genus makes the establishment of directions of chromosome change impossible at present. It is true that diploid numbers of $2n = 48$ and $2n = 50$ are common within different lineages within the genus; however, these numbers are almost always associated with high FN's as clearly demonstrated by the *C. bergi* specimens studied here, which show the largest FN (90) yet found within the genus. Thus, the presence of a $2n = 52$ with relatively low FN in a primitive form such as *C. osvaldoreigi* is relevant because of its rarity, and it is interesting that *C. opimus* (the extant form which is considered to be closely related to the ancestral *Ctenomys* stock; CONTRERAS et al. 1990) is $2n = 26$ and $FN = 48$ (all the autosomes being biarmed); thus, a Robertsonian (fusion/fission) relationship could exist between both species which of course, will have to be proved through banded karyotype comparisons. The complex nature of the interpretation of cytogenetic data in this genus is further demonstrated by the fact that the species which shows the closest karyotypic affinities with *C. osvaldoreigi* and *C. rosendopascuali* is *C. pilarensis* from Paraguay which as noted above, belongs to a different progeny (GIMÉNEZ et al. 1996); its polytypic karyotype is also characterized by $2n = 48-50$ and a low FN (50). This similarity could reflect a common primitive condition for both independent lineages.

Two further problems deserve discussion. First, both species have the simple asymmetrical type of sperm while they belong according to other evidence to a group of forms that have the purportedly primitive character state, i.e., the symmetrical sperm (CONTRERAS 1996; VITULLO et al. 1988; VITULLO and COOK 1991). It is thus tempting to assign *C. osvaldoreigi* or an immediate ancestor, the role of having generated the novel asymmetric sperm. This could explain why the putatively derived species *C. yolandae* and *C. bonettoi* have asymmetrical sperm (complex asymmetrical in the case of the first species); however, the Chilean species of *Ctenomys* also have asymmetrical sperm and their affinities to Argentinian species is obscure. It is at least possible that the asymmetrical sperm condition evolved independently in more than one lineage.

Finally, *C. rosendopascuali* and *C. osvaldoreigi* were studied as part of a project to construct a molecular phylogeny of the genus using analysis of cytochrome b sequences of

mtDNA (MIROL et al. 1995 a, b). Our preliminary results confirm the ancestral nature of *C. osvaldoreigi* but indicate a rather distant relationship with *C. rosendopascuali* which is very close to *C. yolandae* and surprisingly, to *C. bergi*. These results further stress a paradox that is not unique to *Ctenomys*: taxa that speciate rapidly via chromosomal means are probably less readily explainable in evolutionary terms based solely on chromosomal arrangement.

Acknowledgements

This work was partially financed through a British Council-Fundación Antorchas grant to C.J.B. The authors affiliated to the CONICET gratefully acknowledge its continuous support. M.D.G., C.J.B., and C.F.A. were benefited by the Sistema Nacional de Incentivos a la Investigación of the Secretaría de Ciencia y Técnica (Argentina).

Zusammenfassung

Chromosomale Charakterisierung und karyologische Beziehungen zwischen zwei neuen Arten von Ctenomys (Rodentia, Ctenomyidae) aus dem Norden der Provinz Córdoba, Argentinien

Die Karyotypen zweier neu beschriebener *Ctenomys*-Arten aus dem nördlichen Teil der Provinz Córdoba wurden untersucht. *C. osvaldoreigi* ist nur aus der Terra typica der Art in den über 2000 m Seehöhe gelegenen Tälern der Sierras Grandes bekannt. Der Karyotyp bestand aus $2n = 52$ Chromosomen mit $FN = 56$ und umfaßte 22 Paar graduell in der Größe abnehmender telozentrischer Autosomen, ein Paar subtelozentrischer Autosomen (Nr. 8), zwei Paar kleiner metazentrischer Chromosomen sowie ein Paar Geschlechtschromosomen. Bei *C. rosendopascuali* wurden drei Populationen aus den nordöstlichen Ebenen der Provinz Córdoba analysiert, wobei eine der Populationen in der Terra typica der Art lag. Alle Individuen hatten $2n = 52$, aber die FN's der drei Populationen unterschieden sich voneinander. Die Tiere aus Los Mistoles zeigten $FN = 62$; der Karyotyp bestand aus einem Paar großer subtelozentrischer Autosomen, einem mittelgroßen subtelozentrischen Chromosomenpaar (Nr. 8), zwanzig telozentrischen Paaren, drei kleinen metazentrischen Paaren sowie einem Paar Geschlechtschromosomen. Bei den Tieren aus Candelaria war $FN = 64$; der Karyotyp zeigte ein zweites großes subtelozentrisches Chromosomenpaar als Ersatz für ein großes telozentrisches Paar, während der restliche Chromosomenbestand jenem in Los Mistoles ähnlich war. Ein weiteres großes subtelozentrisches Paar kam in der Mar Chiquita-Population vor. FN war daher 66; der restliche Karyotyp unterschied sich jedoch nicht von den Karyotypen der beiden anderen Populationen. Um die neuen Arten einer bekannten Art aus demselben geographischen Raum gegenüberzustellen, wurden vier Populationen von *C. bergi* aus dem nordwestlichen Córdoba karyotypisiert. Alle Individuen zeigten $2n = 48$, $FN = 90$. Die drei bei *C. rosendopascuali* gefundenen Karyotypen sind einander auffällig ähnlich und über relativ einfache Umgruppierungen auch jenem von *C. osvaldoreigi* verwandt, was die morphologische und molekulare Nähe der beiden Arten unterstreicht.

References

- ANDERSON, S.; YATES, T. L.; COOK, J. A. (1987): Notes on Bolivian mammals. 4. The genus *Ctenomys* in the eastern lowlands. *Am. Mus. Novit.* **2891**, 1–20.
- BIDAU, C. J.; GIMENEZ, M. D.; CONTRERAS, J. R. (1996): Especiación cromosómica y la conservación de la variabilidad genética: el caso del género *Ctenomys* (Rodentia, Caviomorpha, Ctenomyidae). *Mendeliana* **12**, 25–37.
- CONTRERAS, J. R. (1995 a): *Ctenomys osvaldoreigi*, una nueva especie de tucu-tucu procedente de las Sierras de Córdoba, República Argentina (Rodentia: Ctenomyidae). *Nótulas Faunísticas* **84**, 1–4.
- CONTRERAS, J. R. (1995 b): Una nueva especie de tucu-tucu procedente de la llanura cordobesa nor-oriental, República Argentina (Rodentia, Ctenomyidae). *Nótulas Faunísticas* **86**, 1–6.

- CONTRERAS, J. R. (1996): Acerca de la distribución geográfica de la morfología espermática en el género *Ctenomys* (Rodentia, Ctenomyidae). *Nóttulas faunísticas* **88**, 1–5.
- CONTRERAS, L. L.; TORRES-MURA, J.; SPOTORNO, A. E. (1990): The largest known chromosome number for a mammal, in a South American desert rodent. *Experientia* **46**, 506–508.
- EVANS, E. P.; BRECKON, G.; FORD, C. E. (1964): An air-drying method for meiotic preparations from mammalian testes. *Cytogenetics* **3**, 289–294.
- FORD, C.; HAMERTON, J. L. A. (1956): A colchicine hypotonic citrate squash sequence for mammalian chromosomes. *Stain Technol.* **31**, 247–251.
- FREITAS, T. R. O. DE (1994): Geographical variation of heterochromatin in *Ctenomys flamarioni* (Rodentia-Octodontidae) and its cytogenetic relationships with other species of the genus. *Cytogenet. Cell Genet.* **67**, 193–198.
- GIMENEZ, M. D.; BIDAÚ, C. J. (1994): A first report of HSRs in chromosome 1 of *Mus musculus domesticus* from South America. *Hereditas* **121**, 291–294.
- GIMENEZ, M. D.; BIDAÚ, C. J.; CONTRERAS, J. R.; DAVIES, Y. E.; ARGUELLES, C. F. (1996): Multiformalidad cromosómica en *Ctenomys* (Rodentia, Ctenomyidae): nuevos cariotipos de especies argentinas. *Reunión de Comunicaciones Científicas y Tecnológicas, Universidad Nacional del Nordeste (Resistencia)* **3**, 29–32.
- GIMENEZ, M. D.; CONTRERAS, J. R.; BIDAÚ, C. J. (1997): Chromosomal variation in *Ctenomys pilarensis*, a recently described species from Eastern Paraguay (Rodentia, Ctenomyidae). *Mammalia* **61**, 385–398.
- HOWELL, W. M.; BLACK, D. A. (1980): Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* **36**, 1014–1015.
- KING, M. (1993): *Species Evolution. The role of chromosome change.* Cambridge: Cambridge Univ. Press.
- LANDE, R. (1985): The fixation of chromosomal rearrangements in a subdivided population with local extinction and colonization. *Heredity* **54**, 323–332.
- MASCHERETTI, S.; MIROL, P. M.; GIMENEZ, M. D.; CONTRERAS, J. R.; BIDAÚ, C. J.; SEARLE, J. B. (1999): Phylogenetics of the speciose and chromosomally variable genus *Ctenomys*, based on mitochondrial cytochrome b sequences. *Biol. J. Linn. Soc.* (in press).
- MASSARANI, A. I.; BARROS, M. A.; ORTELLS, M. O.; REIG, O. A. (1991): Chromosomal polymorphism and small karyotypic differentiation in a group of *Ctenomys* species from central Argentina (Rodentia: Octodontidae). *Genetica* **83**, 131–144.
- MIROL, P. M.; SEARLE, J. B.; GIMENEZ, M. D.; BIDAÚ, C. J.; (1995 a): Molecular correlates of rapidly evolving chromosomes in *Ctenomys* (Rodentia, Ctenomyidae). *Chromosome Research* **3** (Suppl. 1), 80.
- ORTELLS, M. O. (1990): *Biología evolutiva en el género Ctenomys* (Rodentia, Octodontidae). Diss. thesis, Universidad de Buenos Aires.
- ORTELLS, M. O. (1995): Phylogenetic analysis of G-banded karyotypes among the South American subterranean rodents of the genus *Ctenomys* (Caviomorpha: Octodontidae), with special reference to chromosomal evolution and speciation. *Biol. J. Linn. Soc.* **54**, 43–70.
- ORTELLS, M. O.; CONTRERAS, J. R.; REIG, O. A. (1990): New *Ctenomys* karyotypes (Rodentia, Octodontidae) from north-eastern Argentina and from Paraguay confirm the extreme chromosomal multiformality of the genus. *Genetica* **82**, 189–201.
- REIG, O. A. (1984): El significado de los métodos citogenéticos para la distinción y la interpretación de las especies, con especial referencia a los mamíferos. *Rev. Mus. Arg. Cs. Nat. Bernardino Rivadavia* **13**, 19–44.
- REIG, O. A. (1989): Karyotypic repatterning as a triggering factor in cases of explosive speciation. In: *Evolutionary Biology of Transient, Unstable Populations.* Ed. by A. FONTDEVILA. Berlin, New York: Springer-Verlag. Pp. 246–289.
- REIG, O. A.; BUSCH, C.; ORTELLS, M. O.; CONTRERAS, J. R. (1990): An overview of evolution, systematics, population biology, cytogenetics, molecular biology, and speciation in *Ctenomys*. In: *Evolution of Subterranean Mammals at the Organismal and Molecular Levels.* Ed. by E. NEVO and O. A. REIG. New York: Alan R. Liss. Pp. 71–96.
- REIG, O. A.; MASSARANI, A. I.; ORTELLS, M. O.; BARROS, M. A.; TIRANTI, S. I.; DYZENCHAUZ, F. J. (1992): New karyotypes and C-banding patterns of the subterranean rodents of the genus *Ctenomys* (Caviomorpha, Octodontidae) from Argentina. *Mammalia* **56**, 603–623.
- RUEDAS, L. A.; COOK, J. A.; YATES, T. L.; BICKHAM, J. W. (1993): Conservative genome size and rapid chromosomal evolution in the South American tuco-tucos (Rodentia: Ctenomyidae). *Genome* **36**, 449–458.

- SEABRIGHT, M. (1971): A rapid banding technique for human chromosomes. *Lancet* **2**, 971–972.
- SUMNER, A. T. (1972): A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell. Res.* **75**, 304–306.
- VITULLO, A. D.; COOK, J. A. (1991): The role of sperm morphology in the evolution of tuco-tucos, *Ctenomys* (Rodentia: Ctenomyidae): confirmation of results from Bolivian species. *zeitschrift für Säugetierkunde* **56**, 359–364.
- VITULLO, A.; ROLDAN, E. R. S.; MERANI, M. S. (1988): On the morphology of spermatozoa of tuco-tucos, *Ctenomys* (Rodentia: Ctenomyidae): New data and its implications for the evolution of the genus. *J. Zool. (London)* **215**, 675–683.

Authors' addresses: MABEL DIONISIA GIMENEZ, CLAUDIO JUAN BIDAU, CARINA FRANCISCA ARGÜELLES, Laboratorio de Genética Evolutiva, Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones, Felix de Azara 1552, 3300 Posadas, Misiones, Argentina; JULIO RAFAEL CONTRERAS, Museo Argentino de Ciencias Naturales, Av. Angel Gallardo 470, 1405 Buenos Aires, Argentina